UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

WASHINGTON, D.C. 20460

OFFICE OF CHEMICAL SAFETY
AND POLLUTION PREVENTION

MEMORANDUM

DATE: **February 22, 2018**

SUBJECT: **Methyl Isothiocyanate (MITC)**: Cancer Assessment Review Committee's Third Report

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The Cancer Assessment Review Committee (CARC) met on June 21, 2017 to evaluate the carcinogenic potential of methyl isothiocyanate via the inhalation route of exposure in accordance with the EPA's Final Guidelines for Carcinogen Risk Assessment (March, 2005). Attached please find the final Cancer Assessment Document.

EVALUATION OF THE CARCINOGENIC POTENTIAL OF

Methyl Isothiocyanate (MITC)

February 22, 2018

CANCER ASSESSMENT REVIEW COMMITTEE

HEALTH EFFECTS DIVISION

Office of Pesticide Programs

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EXECUTIVE SUMMARY

The Cancer Assessment Review Committee (CARC) reconvened on June 21, 2017 to consider the carcinogenic potential of methyl isothiocyanate (MITC) via the inhalation route of exposure. Previously, MITC was classified as a *Group B2 - probable human carcinogen*, based on statistically significant increases in malignant angiosarcomas in both sexes of the CD-1 mouse in the 2-year drinking water carcinogenicity study. This tumorigenic response is supported by the occurrence of a similar tumor type (malignant hemangiosarcomas) in male Wistar rats. At that time, a low dose extrapolation model was applied to the animal data for the quantification of human risk (Q_1*). In March 1995, an estimated unit risk, Q_1* (mg/kg/day), of 1.98×10^{-1} in human equivalents, was calculated for metam sodium, the major fumigant that rapidly degrades to MITC, based upon angiosarcoma rates in male mice.

In 2004, the Health Effects Division (HED) Science Policy Council (SPC) met to discuss issues related to characterizing the cancer risk of MITC and metam sodium. The SPC concluded that: 1) metam sodium was not genotoxic; 2) there was a concern for chronic exposure to MITC in ambient air; 3) there was also concern that lesions (focal squamous cell metaplasia in the respiratory epithelium of rats at 100 µg/L) seen in the 28-day rat inhalation study could potentially progress to cancer; and 4) the database of acceptable inhalation studies was limited and seven studies were identified as data gaps. In response to these data needs, the MITC Task Force has submitted two new studies addressing HED's requirement for inhalation carcinogenicity studies in rats and mice, as well as a MITC Task Force position paper stating that the highest concentrations in the rat inhalation chronic toxicity/carcinogenicity and mouse inhalation carcinogenicity studies exceeded the maximum tolerated dose (MTD). These submissions are the focus of this report.

In an inhalation carcinogenicity study in rats, MITC was administered to 60 Sprague Dawley [Crl:CD(SD)] rats/sex/dose by whole-body inhalation at nominal concentrations of 0, 0.5, 5 and 20 ppm (equivalent to 0, 0.001, 0.015, or 0.060 mg/L)[1] diluted with filtered air for 6 h/day, 5 days/week for 104 weeks.

Male and female rats had statistically significant trends and showed significant pair-wise comparisons for nasal carcinomas at the high dose (20 ppm; 0.06 mg/L) compared to the controls.

The CARC concluded that the high test concentration was adequate and not excessively toxic for evaluating carcinogenicity, based on the following observations:

1. There was no clear evidence that the high concentration resulted in systemic toxicity or otherwise confounded observed results.
2. There were no statistically significant differences in early mortality among the treatment groups throughout the study.
3. Given the high metastatic rate of the nasal tumors (and association with mortality), the CARC could not exclude the impact of tumors on body weight decreases and lung weight increases and clinical signs such as rales, labored respiration, and nasal discharge. Accordingly, it was concluded that tumor-associated changes in body or

[1]Analytical concentrations were 0, 0.5, 4.83 and 19.87 ppm; equivalent to the above mg/L concentrations.

organ weight were not a sufficient reason to consider the high concentration to be excessively toxic.
4. The majority of the non-neoplastic findings at the high concentration were mild to moderate severity, and there was no clear evidence of a distinct biological process leading to increased mortality or other overt toxicity at the high concentration.
5. The data for the high concentration were consistent with a robust irritant effect, but there was no overwhelming necrosis seen at the tumorigenic level (*e.g.,* generally mild to moderate necrosis was only seen in 5 male and 3 female rats at Nasal Level I).
6. Neither the study pathologist nor the peer review pathologist noted that 0.06 mg/L was an excessive concentration that may have confounded study interpretation.

The CARC concluded that the nasal tumors in male and female rats are treatment-related.

In an inhalation carcinogenicity study in mice, MITC was administered to 50 CD-1 mice/sex/concentration as a vapor by whole-body inhalation at target concentrations of 0, 1, 5, and 15 ppm (equivalent to 0.003, 0.015, or 0.045 mg/L)[2] for 6 h/day, 5 days/week for up to 78 weeks. The high concentration of MITC was considered adequate and not excessively toxic for evaluating carcinogenicity based on decreases in body weight and food consumption in both sexes and non-neoplastic, microscopic findings in the nasal levels of the respiratory tract at 0.045 mg/L.

The CARC concluded there was no evidence of carcinogenicity in male or female mice.

In accordance with the EPA's Final Guidelines for Carcinogen Risk Assessment (March 2005), the CARC classified MITC via the inhalation route as "Likely to be Carcinogenic to Humans" based on treatment-related nasal tumors in both sexes in the rat. The CARC recommended a linear low-dose extrapolation model (Q1*) for human cancer risk assessment.

2. Analytical concentrations were 0, 1.03, 5.06 and 15.11 ppm; equivalent to the above mg/L concentrations.

I. BACKGROUND

Methyl isothiocyanate (MITC), which has a high vapor pressure, is the primary toxic degradate of concern for the non-selective pre-plant or postharvest soil fumigants, metam sodium (sodium N-methyldithiocarbamate), metam potassium (potassium N-methyldithiocarbamate) and dazomet (tetrahydro-3,5-dimethyl-2H-1, 3,5-thiadiazine-2-thione). Metam sodium was presented to the Cancer Peer Review Committee (CPCR) in 1995.

At this meeting, the CPRC concluded that metam sodium should be classified as a *Group B2 - probable human carcinogen*, based on statistically significant increases in malignant angiosarcomas in both sexes of the CD-1 mouse, and a similar tumor type (malignant hemangiosarcomas) in male Wistar rats (HED Document No. 011541). In both the mouse and rat studies, metam sodium was administered in the drinking water. The CPRC recommended that for the purpose of risk characterization, a low dose extrapolation model be applied to the animal data for the quantification of human risk (Q_1*), based on the total incidence of angiosarcomas in male mice, at all sites combined. In March 1995, an estimated unit risk, Q_1* (mg/kg/day), of 1.98×10^{-1} in human equivalents, was calculated for metam sodium based upon angiosarcoma rates in male mice (Memo, B. Fisher, 3/10/95, TXR No. 0012954).

The Pathology Working Group (PWG) review of hemangiomatous lesions in the male Wistar rats (MRID 47067501) from the two-year chronic toxicity/carcinogenicity drinking water study of metam sodium (MRID 43275802) was the subject of the CARC re-evaluation of the cancer classification of metam sodium based on the PWG review in 2009 (Kidwell, 2009; TXR No. 0055107) as well as a subchronic inhalation toxicity study in rats (MRID 45314802) and the *in vitro* cytogenetic study in human lymphocytes (MRID 44276402). Based on this re-evaluation and in accordance with the *EPA's Final Guidelines for Carcinogen Risk Assessment* (March 2005), the CARC classified metam sodium as **"Likely to be Carcinogenic to Humans."** Consequently, the low dose extrapolation model which was applied to the animal data for the quantification of human risk (Q_1*), based on the total incidence of angiosarcomas in male mice at all sites combined remains in place.

Members of the metam sodium risk assessment team and the Health Effects Division (HED) Science Policy Council (SPC) met on August 10, 2004 to discuss issues related to characterizing the cancer risk of MITC and metam sodium. From these deliberations, the SPC concluded the following:

1. Metam sodium shows no evidence of genotoxicity;
2. Chronic exposure to MITC in ambient air is possible.
3. Based on the results from a 28-day rat inhalation study on MITC, showing an increase in focal squamous cell metaplasia in the respiratory epithelium of rats at 100 µg/L, there is a concern that this lesion could potentially progress and result in cancer.

When the Reregistration Eligibility Decision was completed in 2009 (Smith, 2009; DP Barcode D357118), the database of acceptable inhalation studies was limited, and seven studies were identified as data gaps. In response to these data needs, the MITC Task Force submitted three new studies addressing HED's requirement for inhalation carcinogenicity studies in rats and mice. On June 21, 2017, CARC reconvened to evaluate the following new submissions:

1. Kirkpatrick, D.T. (2015) An 18-month whole-body inhalation carcinogenicity study of methyl isothiocyanate (MITC) in mice. WIL Research, Ashland, OH. Laboratory Project ID: WIL-824014, November 13, 2015. MRID 49779601. Unpublished.
2. Kirkpatrick, D.T. (2015) A combined inhalation chronic toxicity and 24-month carcinogenicity study of methyl isothiocyanate (MITC) in rats. WIL Research, Ashland, OH. Laboratory Study Number WIL-824015, November 20, 2015. MRID 49779602. Unpublished.
3. Hauswirth, J.W., Jonynas, A., and Piccirillo, V.J. (2015) Methyl isothiocyanate: Maximum tolerated dose exceedance. MITC Task Force. Project ID: 1015-004-01, November 16, 2015. MRID 49779603. Unpublished.

II. EVALUATION OF CARCINOGENICITY STUDIES

1. Combined chronic toxicity/carcinogenicity study in rats

Citation: Kirkpatrick, D.T. (2015) A combined inhalation chronic toxicity and 24-month carcinogenicity study of methyl isothiocyanate (MITC) in rats. WIL Research, Ashland, OH. Laboratory Study Number WIL-824015, November 20, 2015. MRID 49779602. Unpublished.

A. Experimental Design

In a combined chronic toxicity/carcinogenicity study (MRID 49779602), methyl isothiocyanate (MITC; Lot # 56198PJV; 97.2-99.7% a.i.) was administered to 60 Sprague Dawley [Crl:CD(SD)] rats/sex/dose by whole-body inhalation at nominal concentrations of 0, 0.5, 5 and 20 ppm (equivalent to 0, 0.001, 0.015, or 0.060 mg/L)[3] diluted with filtered air for 6 h/day, 5 days/week for 104 weeks. Blood smears were prepared from all Carcinogenicity Phase animals euthanized *in extremis* (if possible) and from all surviving animals at the scheduled necropsy (study week 104). Complete necropsies were performed on all animals and selected organs were weighed at the scheduled necropsies. Selected tissues were examined microscopically from all animals found dead or euthanized *in extremis* and from all animals in the control and high-concentration groups at the scheduled necropsies. A histopathology peer review was conducted for both the chronic toxicity and carcinogenicity phases of testing. The microscopic pathology findings presented for this study represent a consensus between the study pathologist and the peer review pathologist.

B. Survival

Survival data are summarized in Tables 1 (males) and 2 (females). As shown, there were no statistically significant disparities among the treatment groups in males or females. It is of note, however, that over the course of the study, the cause of death for 18 and 23% of high concentration males and females, respectively, was nasal tumors.

[3]Analytical concentrations were 0, 0.5, 4.83 and 19.87 ppm; equivalent to the above mg/L concentrations.

Table 1. MITC – Sprague-Dawley [Crl:CD(SD)] Rat Study (MRID No. 49779602)

Male Mortality Rates[+] and Cox or Generalized K/W Test Results

	Weeks					
Dose (ppm)	**1-26**	**27-52**	**52[i]**	**53-78**	**79-105[f]**	**Total**
0	1/60	7/59	7/52	10/45	21/35	39/53 (74)
0.5	2/60	5/58	9/53	13/44	17/31	37/51 (73)
5.0	1/60	5/59	9/54	13/45	18/32	37/51 (73)
20.0	2/60	4/58	7/54	11/47	22/36	39/53 (74)

Data were extracted from Brunsman, L.L. (TXR No. 0057612; May 24, 2017).
+Number of animals that died during the interval/Number of animals alive at the beginning of the interval.
[i]Interim sacrifice at week 52.
[f]Final sacrifice at weeks 104-105.
( ) Percent.
Note:
Time intervals were selected for display purposes only.
Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If [*], then $p < 0.05$. If [**], then $p < 0.01$.

Table 2. MITC – Sprague-Dawley [Crl:CD(SD)] Rat Study (MRID No. 49779602)

Female Mortality Rates[+] and Cox or Generalized K/W Test Results

Weeks						
Dose (ppm)	**1-26**	**27-52**	**52[i]**	**53-78**	**79-105[f]**	**Total**
0	0/60	4/60	10/56	10/46	20/36	34/50 (68)
0.5	2/60	3/58	8/55	12/47	18/35	35/52 (67)
5.0	0/60	5/60	9/55	10/46	17/36	32/51 (63)
20.0	0/60	4/60	10/56	14/46	17/32	35/50 (70)

Data were extracted from Brunsman, L.L. (TXR No. 0057612; May 24, 2017).
+Number of animals that died during the interval/Number of animals alive at the beginning of the interval.
[i]Interim sacrifice at week 52.
[f]Final sacrifice at weeks 104-105.
( ) Percent.
Note: Time intervals were selected for display purposes only.
Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

C. Tumor Analyses (Males)

i. Discussion of the Nasal Tumor Data

The statistical analyses of the tumors in male rats were based upon Fisher's Exact Test and the Exact Test for Trend and are presented in **Tables 3, 4, 5, and 6**. As shown in **Table 3**, male rats treated with 20 ppm MITC had significant trends for nasal adenomas at $p < 0.05$, and for papillomas and squamous cell carcinomas at $p < 0.01$. Similarly, pairwise comparisons were significant for papillomas ($p < 0.05$) and squamous cell carcinomas ($p < 0.01$). All nasal tumor carcinomas combined showed a significant trend and pair wise comparison at $p < 0.01$. Similarly, all nasal tumors (adenomas, papillomas, squamous cell papillomas, anaplastic carcinomas, squamous cell carcinomas, and carcinosarcomas) combined were significantly increased at $p < 0.01$ (both trend and pair-wise comparison).

Table 3. MITC – Sprague-Dawley [Crl:CD(SD)] Rat Study (MRID No. 49779602)

Male Nasal Tumor Rates[+] and Fisher's Exact Test and Exact Trend Test Results

Dose (ppm)	**0**	**0.5**	**5.0**	**20.0**
(mg/L)	**0**	**0.0015**	**0.015**	**0.06**
Adenomas (%) P =	0/60 (0) 0.0150*	0/60 (0) 1.0000	0/60 (0) 1.0000	3[a]/60 (5) 0.1219
Sebaceous Cell Adenomas (%) P =	1[b]/60 (2) 0.1872	1/60 (2) 0.7521	0/60 (0) 1.0000	0/60 (0) 1.0000
Papillomas (%) P =	0/60 (0) 0.0002**	0/60 (0) 1.0000	0/60 (0) 1.0000	6[c]/60 (10) 0.0137*
Squamous Cell Papillomas (%) P =	0/60 (0) 0.2500	0/60 (0) 1.0000	0/60 (0) 1.0000	1[d]/60 (2) 0.5000
Anaplastic Carcinomas (%) P =	0/60 (0) 0.0617	0/60 (0) 1.0000	0/60 (0) 1.0000	2[e]/60 (3) 0.2479
Squamous Cell Carcinomas (%) P =	0/60 (0) 0.0000**	0/60 (0) 1.0000	0/60 (0) 1.0000	15[f]/60 (25) 0.0000**
Carcinosarcomas (%) P =	0/60 (0) 0.2500	0/60 (0) 1.0000	0/60 (0) 1.0000	1[g]/60 (2) 0.5000
All Nasal Carcinomas Combined (Anaplastic Carcinoma, Squamous Cell Carcinoma and Carcinosarcoma) (%) P =	0/60 (0) 0.0000**	0/60 (0) 1.0000	0/60 (0) 1.0000	17[h]/60 (28) 0.0000**
All Nasal Tumors Combined (Adenomas, Papillomas, and Carcinomas) (%) P =	1/60 (2) 0.0000**	1/60 (2) 0.7521	0/60 (0) 1.0000	25[i]/60 (42) 0.0000**

Data were extracted from Brunsman, L.L. (TXR No. 0057612; May 24, 2017).

+Number of tumor bearing animals/Number of animals examined, excluding those that died or were sacrificed before week 52.

[a]First adenoma observed at week 104 in the 20 ppm dose group.

[b]First sebaceous cell adenoma observed at week 103 in the control group.

[c]First papilloma observed at week 52 in the 20 ppm dose group.

[d]First squamous cell papilloma observed at week 104 in the 20 ppm dose group.
[e]First anaplastic carcinoma observed at week 61 in the 20 ppm dose group.
[f]First squamous cell carcinoma observed at week 39 in the 20 ppm dose group.
[g]First carcinosarcoma observed at week 98 in the 20 ppm dose group.
[h] First nasal carcinoma observed at week 39 in the 20 ppm dose group.
[i]Three animals in the 20 ppm dose group had multiple tumor types**.** Data for "All Combined Nasal Carcinomas" was based on the animal count rather than tumor quantity; if an animals had multiple tumor sites, it was only counted once
Note: Significance of trend denoted at control. Significance of pair-wise comparison with control denoted at dose level. If *, then $p < 0.05$. If **, then $p < 0.01$.

ii. Historical control information

No historical control data were available for chronic inhalation studies with Sprague Dawley rats either from WIL Research or Charles River Laboratories in the U.S., Canada or Scotland or the National Toxicology Program. All of the nasal tumors were reported by the investigators to be "rare" tumors.

Table 4 summarizes the male rat lung tumor data and shows no significant trend or pair-wise comparison for lung papillomas, anaplastic carcinomas, carcinomas or all lung tumors combined.

Table 4. MITC – Sprague-Dawley [Crl:CD(SD)] Rat Study

(MRID No. 49779602)

Male Lung Tumor Rates[+] and Fisher's Exact Test and Exact Trend Test Results

Dose (ppm)	**0**	**0.5**	**5.0**	**20.0**
(mg/L)	**0**	**0.0015**	**0.015**	**0.06**
Papillomas	0/53	0/51	0/51	1[a]/53
(%)	(0)	(0)	(0)	(2)
P =	0.2548	1.0000	1.0000	0.5000
Anaplastic Carcinomas	0/53	0/51	0/51	1[b]/53
(%)	(0)	(0)	(0)	(2)
P =	0.2548	1.0000	1.0000	0.5000
Carcinomas	0/53	1[c]/51	0/51	0/53
(%)	(0)	(2)	(0)	(0)
P =	0.5000	0.4904	1.0000	1.0000
Combined	0/53	1/51	0/51	2/53
(%)	(0)	(2)	(0)	(4)
P =	0.1109	0.4904	1.0000	0.2476

Data were extracted from Brunsman, L.L. (TXR No. 0057612; May 24, 2017).
+Number of tumor bearing animals/number of animals examined, excluding those that died or were sacrificed before week 53.
[a]First papilloma observed at week 104 in the 20 ppm dose group.
[b]First anaplastic carcinoma observed at week 61 in the 20 ppm dose group.
[c]First carcinoma observed at week 98 in the 0.5 ppm dose group.

Note: Significance of trend denoted at control. Significance of pair-wise comparison with control denoted at dose level. If *, then $p < 0.05$. If **, then $p < 0.01$.

Based on the pathology report, there was only one primary lung neoplasm (papilloma) observed in a single male rat (no. 4151) in the 20 ppm group at the scheduled necropsy. All other lung tumors in males (and females) were considered secondary to nasal tumors or other incidental tumor types. Similarly, no significant trend or pair-wise comparison was seen for male skin keratoacanthoma, sebaceous cell adenomas squamous cell papillomas, basal cell carcinomas, or squamous cell carcinomas of the skin when considered individually or combined **(Table 5).**

Table 5. MITC – Sprague-Dawley [Crl:CD(SD)] Rat Study (MRID No. 49779602)

Male Skin and Subcutis Carcinoma Tumor Rates[+] and Fisher's Exact Test and Exact Trend Test Results

Dose (ppm)	**0**	**0.5**	**5.0**	**20.0**
(mg/L)	**0**	**0.0015**	**0.015**	**0.06**
Skin Keratoacanthomas (%) P =	1[a]/53 (2) 0.2548	0/51 (0) 1.0000	0/51 (0) 1.0000	0/53 (0) 1.0000
Skin Sebaceous Cell Adenomas (%) P =	1[b]/53 (2) 0.1896	1[b]/51 (2) 0.7427	0/51 (0) 1.0000	0/53 (0) 1.0000
Skin Squamous Cell Papillomas (%) P =	0/53 (0) 0.1896	0/51 (0) 1.0000	1/51 (2) 0.4904	1[c]/53 (2) 0.5000
Skin Basal Cell Carcinomas (%) P =	1[d]/53 (2) 0.2548	0/51 (0) 1.0000	0/51 (0) 1.0000	0/53 (0) 1.0000
Skin Squamous Cell Carcinomas (%) P =	0/53 (0) 0.2548	0/51 (0) 1.0000	0/51 (0) 1.0000	1[e]/53 (2) 0.5000
Subcutis Squamous Cell Carcinomas (%) P =	0/53 (0) 0.0640	0/51 (0) 1.0000	0/51 (0) 1.0000	2[f]/53 (4) 0.2476
Subcutis Carcinomas (%) P =	0/53 (0) 0.5000	1[g]/51 (2) 0.4904	0/51 (0) 1.0000	0/53 (0) 1.0000
Combined (%) P =	3/53 (6) 0.2136	2/51 (4) 0.8064	1/51 (2) 0.9363	4/53 (8) 0.5000

Data were extracted from Brunsman, L.L. (TXR No. 0057612; May 24, 2017).
+Number of tumor bearing animals/Number of animals examined, excluding those that died or were sacrificed before week 53.
[a]First skin keratoacanthoma observed at week 56 in the control group.
[b]First skin sebaceous cell adenomas observed simultaneously at the final sacrifice in the control and 0.5 ppm dose groups.
[c]First skin squamous cell papilloma observed at week 81 in the 20 ppm dose group.
[d]First skin basal cell carcinoma observed at the final sacrifice in the control group.
[e]First skin squamous cell carcinoma observed at week 92 in the 20 ppm dose group.
[f]First subcutis squamous cell carcinoma observed at week 85 in the 20 ppm dose group.
[g]First subcutis carcinoma observed at week 98 in the 0.5 ppm dose group.
Note: Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

Although pituitary adenomas (males and females) or carcinomas (males only) were also seen in this study, the tumor rates for either the adenomas or carcinomas in the male rats or adenomas in the females were lower than the filtered air control group at all concentrations. These pituitary neoplasms are common background tumors in aging rats. It was, therefore, concluded that these findings were not treatment-related.

D. Tumor Analyses (Females)

The statistical analyses of the tumors in female rats were based upon Fisher's Exact Test and the Exact Test for Trend; these data are summarized in **Tables 6, 7, and 8**.
Female rats had significant trends, and significant pair-wise comparisons of the high dose group with the controls, for nasal squamous cell carcinomas, and when nasal anaplastic carcinomas and squamous cell carcinomas were combined, all at $p < 0.01$ **(Table 6).**

Table 6. MITC – Sprague-Dawley [Crl:CD(SD)] Rat Study (MRID No. 49779602)

Female Nasal Tumor Rates[+] and Fisher's Exact Test and Exact Trend Test Results

Dose (ppm)	0	0.5	5.0	20.0
(mg/L)	0	0.0015	0.015	0.06
Anaplastic Carcinomas	0/60	0/60	0/60	2[a]/60
(%)	(0)	(0)	(0)	(3)
P =	0.06172	1.00000	1.00000	0.24790
Squamous Cell Carcinomas				
(%)	0/60	0/60	0/60	15[b]/60
	(0)	(0)	(0)	(25)
P =	0.00000**	1.00000	1.00000	0.00001**
Combined	0/60	0/60	0/60	16[c]/60
(%)	(0)	(0)	(0)	(27)
P =	0.00000**	1.00000	1.00000	0.00000**

Data were extracted from Brunsman, L.L. (TXR No. 0057612; May 24, 2017).

+Number of tumor bearing animals/Number of animals examined, excluding those that died or were sacrificed before week 52.

[a]First anaplastic carcinoma observed at week 69 in the 20 ppm dose group.

[b]First squamous cell carcinoma observed at week 48 in the 20 ppm dose group.

[c]One animal in the 20 ppm dose group had both an anaplastic carcinoma and a squamous cell carcinoma.

Note: Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

For lung tumors, there was a significant trend ($p < 0.01$) at 20 ppm for all lung carcinomas in female rats when these malignant tumors were combined (**Table 7**). However, all of these carcinomas in females were flagged in the pathology report as metastatic, likely from the nasal cavity. Removing these metastatic tumors shows no distinct treatment effect in the lung.

Table 7. MITC – Sprague-Dawley [Crl:CD(SD)] Rat Study (MRID No. 49779602)

Female Lung Tumor Rates[+] and Fisher's Exact Test and Exact Trend Test Results

Dose (ppm)	0	0.5	5.0	20.0
mg/L	**0**	**0.0015**	**0.015**	**0.06**
Anaplastic Carcinomas (%) P =	0/50 (0) 0.2463	0/52 (0) 1.0000	0/51 (0) 1.0000	1[a]/50 (2) 0.5000
Squamous Cell Carcinomas (%) P =	0/50 (0) 0.0598	0/52 (0) 1.0000	0/51 (0) 1.0000	2[b]/50 (4) 0.2475
Carcinosarcomas (%) P =	1[c]/50 (2) 0.2463[n]	0/52 (0) 1.0000	0/51 (0) 1.0000	0/50 (0) 1.0000
Adenocarcinomas (%) P =	0/50 (0) 0.1841	0/52 (0) 1.0000	1/51 (2) 0.5050	1[d]/50 (2) 0.5000
Sebaceous Cell Carcinomas (%) P =	0/50 (0) 0.2463	0/52 (0) 1.0000	0/51 (0) 1.0000	1[e]/50 (2) 0.5000
Combined (%) P =	1/50 (2) 0.0064**	0/52 (0) 1.0000	1/51 (2) 0.7574	5/50 (10) 0.1022

Data were extracted from Brunsman, L.L. (TXR No. 0057612; May 24, 2017).
+Number of tumor bearing animals/Number of animals examined, excluding those that died or were sacrificed before week 53.
[n]Negative trend.
[a]First anaplastic carcinoma observed at week 91 in the 20 ppm dose group.
[b]First squamous cell carcinoma observed at week 52 in the 20 ppm dose group.
[c]First carcinosarcoma observed at week 75 in the control group.
[d]First adenocarcinoma observed at week 79 in the 20 ppm dose group.
[e]First sebaceous cell carcinoma observed at week 61 in the 20 ppm dose group.
Note: Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

As shown in **Table 8**, no significant trends or pair-wise comparisons were found for skin tumors when analyzed individually or combined. Significant ($p < 0.05$) trends but no pair-wise comparisons were obtained for skin squamous cell carcinomas and subcutis adenocarcinomas; the latter tumor type showed a negative trend.

Table 8. MITC – Sprague-Dawley [Crl:CD(SD)] Rat Study (MRID No. 49779602)

Female Skin and Subcutis Carcinoma Tumor Rates+ and Fisher's Exact Test and Exact Trend Test Results

Dose (ppm)	0	0.5	5.0	20.0
mg/L	0	0.0015	0.015	0.06
Skin Squamous Cell Papillomas	1[a]/60	0/60	0/60	0/60
(%)	(2)	(0)	(0)	(0)
P =	0.2500[n]	1.0000	1.0000	1.0000
Skin Squamous Cell Carcinomas	1[b]/60	0/60	0/60	3/60
(%)	(2)	(0)	(0)	(5)
P =	0.0493*	1.0000	1.0000	0.3093
Skin Adenocarcinomas	5/60	2/60	4/60	5[c]/60
(%)	(8)	(3)	(7)	(8)
P =	0.2822	0.9430	0.7547	0.6285
Subcutis Sebaceous Cell Adenomas	1[d]/60	0/60	0/60	0/60
(%)	(2)	(0)	(0)	(0)
P =	0.2500[n]	1.0000	1.0000	1.0000
Subcutis Adenomas	3[e]/60	1/60	0/60	1/60
(%)	(5)	(2)	(0)	(2)
P =	0.2571[n]	0.9406	1.0000	0.9406
Subcutis Anaplastic Carcinomas	0/60	0/60	0/60	1[f]/60
(%)	(0)	(0)	(0)	(2)
P =	0.2500	1.0000	1.0000	0.5000
Subcutis Squamous Cell Carcinomas	0/60	0/60	0/60	1[g]/60
(%)	(0)	(0)	(0)	(2)
P =	0.2500	1.0000	1.0000	0.5000
Subcutis Carcinosarcomas	1[h]/60	0/60	0/60	0/60
(%)	(2)	(0)	(0)	(0)
P =	0.2500[n]	1.0000	1.0000	1.0000
Subcutis Adenocarcinomas	7/60	9[i]/60	6/60	2/60
(%)	(12)	(15)	(10)	(3)
P =	0.0151*	0.3946	0.7208	0.9839
Subcutis Sebaceous Cell Carcinomas	0/60	0/60	0/60	1[j]/60
(%)	(0)	(0)	(0)	(2)
P =	0.2500	1.0000	1.0000	0.5000
Combined	14[k]/60	12/60	7[m]/60	14/60
(%)	(23)	(20)	(12)	(23)
[n]Negative trend. P =	0.3312	0.7466	0.9736	0.5853

Data were extracted from Brunsman, L.L. (TXR No. 0057612; May 24, 2017).
+Number of tumor bearing animals/Number of animals examined, excluding those that died or were sacrificed before week 52.
[a]First skin squamous cell papilloma observed at week 104 in the control group.
[b]First skin squamous cell carcinoma observed at week 52 in the control group.
[c]First skin adenocarcinoma observed at week 39 in the 20 ppm dose group.
[d]First subcutis sebaceous cell adenoma observed at week 104 in the control group.
[e]First subcutis adenoma observed at week 94 in the control group.
[f]First subcutis anaplastic carcinoma observed at week 69 in the 20 ppm dose group.
[g]First subcutis squamous cell carcinoma observed at week 76 in the 20 ppm dose group.
[h]First subcutis carcinosarcoma observed at week 75 in the control group.
[i]First subcutis adenocarcinoma observed at week 47 in the 0.5 ppm dose group.
[j]First subcutis sebaceous cell carcinoma observed at week 61 in the 20 ppm dose group.

[k]Five animals in the control group had multiple tumors.
[m]Three animals in the 5 ppm dose group had multiple tumors.

Note: Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If [*], then $p < 0.05$. If [**], then $p < 0.01$

E. Non-Neoplastic Lesions

Non-neoplastic lesions are presented in **Tables 9 (males) and 10 (females)**. As shown, extensive non-neoplastic findings were observed in the nasal cavity (Levels I through VI) as well as the larynx, trachea, lungs, olfactory bulbs and eyes at the high concentration for males and females. At **20 ppm**, hyperplasia in the nasal region (respiratory epithelium, squamous epithelium, or transitional epithelium) occurred in the majority of high concentration male rats [respiratory epithelium (60; 100%) and squamous epithelium (54, 90%)] and female rats [respiratory epithelium (59; 98%), squamous epithelium (55; 92%) or transitional epithelium (47; 78%)]. Similarly, metaplasia (respiratory and squamous epithelium) occurred in all 60 M and in 58 and 60 F, respectively. Both of these tissue transformations are consistent with a chronic active inflammation which was noted in 59/60 M and 59/60 F high concentration rats. In the larynx, epithelial hyperplasia was noted in 29 (48%) M and 32 (53%) F; squamous metaplasia was also seen in all males and females at the high concentration. Similar evidence of hyperplastic respiratory epithelium was seen in the trachea and lungs of the majority of high concentration males and females.

At **5 ppm**, 50, 10, and 59 M and 44, 16 and 52 F showed hyperplasia of the respiratory epithelium, squamous epithelium and transitional epithelium, respectively in the nasal region, along with 38 M and 19 F with squamous metaplasia. Epithelial hyperplasia and squamous metaplasia were also note in the larynx of the 5 ppm-group males (32 and 28) and females (37 and 12).

At **0.5 ppm**, the incidence of respiratory epithelium, squamous epithelium and transitional epithelium, in the nasal region was comparable to controls for males; only the incidence of transitional epithelium hyperplasia was higher than the controls for the females (40 vs 23 in control rats). Epithelial hyperplasia and squamous metaplasia were also note in the larynx of the 0.5 ppm-group males (17 and 6 vs. 3 and 0 in control male rats, respectively) and females (28 and 7 vs. 8 and 1 in control female rats, respectively).

The increased incidence of hyperplasia, which was seen in the air controls in the nasal regions of both sexes of rats, was likely due to environmental conditions/ handling and, therefore, did not likely influence the outcome of the study.

Table 9. MITC – Sprague-Dawley [Crl:CD(SD)] Rat Study (MRID No. 49779602, 49779603)

<u>Male</u> incidence of selected non-neoplastic microscopic respiratory tract findings treated by whole-body inhalation

Concentration (mg/L)	0	0.0015	0.015	0.060
ppm	0	0.5	5.0	20
# animals	60	60	60	60
Nasal (all levels combined)				
Atrophy, Bowman's glands	0	0	28	57
Atrophy, olfactory epithelium	0	0	0	53
Atrophy, olfactory nerve bundle	0	0	32	58
Collapse, dorsal meatus	0	0	0	5
Degeneration, olfactory epithelium	4	5	47	33
Dilatation, Bowman's glands	0	0	0	13
Dilatation, lumen	1	2	4	21
Exudate, inflammatory	21	20	33	60
Hyperplasia, Bowman's glands	0	0	0	2
Hyperplasia, respiratory epithelium /	39	34	50	60
Hyperplasia, squamous epithelium	8	3	10	54
Hyperplasia, transitional epithelium	47	45	59	44
Inflammation, chronic-active	44	31	53	59
Metaplasia, respiratory epithelium	0	1	3	60
Metaplasia, squamous	8	20	38	60
Mineralization	0	0	1	9
Necrosis squamous epithelium	0	0	0	5
Larynx				
Erosion	0	0	0	3
Exudate, inflammatory	15	12	12	25
Hyperplasia, epithelial	3	17	32	29
Metaplasia, squamous	0	6	28	60
Regeneration, respiratory epithelium	0	0	0	10
Trachea				
Hyperplasia, respiratory epithelium	0	0	1	41
Inflammation, mixed cell	0	0	1	10
Metaplasia, squamous	0	0	0	3
Regeneration, respiratory	0	1	1	23
Lung				
Exudate, luminal	1	0	0	29
Fibrosis	0	0	0	39
Fibrosis, pleural	0	0	2	6
Hyperplasia, mucous cell	0	1	0	5
Hyperplasia, respiratory epithelium	0	0	0	45

Inflammation, chronic	0	0	0	18
Macrophages, alveolar	20	23	26	44
Metaplasia, squamous	0	0	0	12
Regeneration, respiratory	0	0	0	41
Ulceration	0	0	0	29

a Data were obtained from Table 5 on pages 10-12 of MRID 49779603 and Text Tables 16, 18, 19, and 20 on pages 89, 97, 99, and 100-101, respectively, of MRID 49779602.

Table 10. MITC – Sprague-Dawley [Crl:CD(SD)] Rat Study (MRID No. 49779602, 49779603)

Female incidence of selected non-neoplastic microscopic respiratory tract findings treated by whole-body inhalation

Concentration (mg/L)	0	0.0015	0.015	0.060
ppm	0	0.5	5	20
# animals	60	60	60	60
Nasal (all levels combined)				
Atrophy, Bowman's glands	0	1	23	56
Atrophy, olfactory epithelium	0	1	2	49
Atrophy, olfactory nerve bundle	0	1	34	56
Collapse, dorsal meatus	0	0	0	3
Degeneration, olfactory epithelium	1	5	43	46
Dilatation, Bowman's glands	0	0	0	20
Dilatation, lumen	0	0	0	13
Exudate, inflammatory	11	10	21	60
Hyperplasia, Bowman's glands	0	0	0	0
Hyperplasia, respiratory epithelium /	24	26	44	59
Hyperplasia, squamous epithelium	11	3	16	55
Hyperplasia, transitional epithelium	23	40	52	47
Inflammation, chronic-active	29	30	51	59
Metaplasia, respiratory epithelium	0	1	4	58
Metaplasia, squamous	3	2	19	60
Mineralization	0	0	1	10
Necrosis squamous epithelium	0	0	2	3
Larynx				
Erosion	0	0	0	1
Exudate, inflammatory	4	4	7	26
Hyperplasia, epithelial	8	28	37	32
Metaplasia, squamous	1	7	12	60
Regeneration, respiratory epithelium	0	0	0	10
Trachea				
Hyperplasia, respiratory epithelium	0	0	1	32
Inflammation, mixed cell	2	1	3	3

Metaplasia, squamous	0	0	0	4
Regeneration, respiratory	0	0	0	3
Lung				
Exudate, luminal	1	0	2	20
Fibrosis	2	2	0	47
Fibrosis, pleural	0	0	1	1
Hyperplasia, mucous cell	1	1	1	4
Hyperplasia, respiratory epithelium	0	0	0	44
Inflammation, chronic	0	0	1	14
Macrophages, alveolar	29	20	18	41
Metaplasia, squamous	0	0	0	4
Regeneration, respiratory	0	0	0	46
Ulceration	0	0	0	32

a Data were obtained from Table 5 on pages 10-12 of MRID 49779603 and Text Tables 16, 18, 19, and 20 on pages 89, 97, 99, and 100-101, respectively, of MRID 49779602.

F. Adequacy of dosing for carcinogenicity assessment

In addition to the two cancer studies, the registrant also submitted a third document claiming that the high concentration (20 ppm MITC) used in the chronic rat toxicity and 24-month inhalation carcinogenicity study was in excess of the maximum tolerated dose (MTD) and, therefore, not appropriate for tumor analysis (MRID 49779603). Based on the evaluation of the toxicity seen in this study, the CARC found the following toxic effects at the tumorigenic concentration, **0.060 mg/L**:

Clinical observations: Increased incidence of rales, labored respiration, clear and red nasal discharge, red material around the nose (males only), and left and right eye opacities. Treatment-related palpable masses on or near the nose were found and were associated with neoplastic findings observed in this area.

Body weights: As shown in **Table 11,** body weights were decreased consistently throughout treatment by 8-34% in the males, and by 6-22% in the females at 0.060 mg/L. Similarly, cumulative body weight gains were generally decreased throughout the study, with overall (Weeks 0-103) body weight gains decreased by 46% and 27% in the males and females, respectively. Decreased food consumption values were generally observed throughout treatment that correlated with the decreased body weights. No effects on body weight, body weight gain, or food consumption were observed at lower concentrations.

Table 11. MITC – Sprague-Dawley [Crl:CD(SD)] Rat Study (MRID No. 49779602, 49779603)
Mean (±SD) body weights and body weight gains (g) treated by whole-body inhalation

Concentration (mg/L)	0	0.0015	0.015	0.060
ppm	0	0.5	5	20
Week	Males			
0	244±16.8	245±19.5	244±18.2	225±16.7** (↓8)
13	551±61.1	565±61.3	574±55.3	462±49.3** (↓16)
53	724±84.8	745±90.0	755±86.1	536±73.1** (↓26)
79	757±106.6	750±113.1	812±99.4	512±89.8** (↓32)
103	720±79.3	708±83.1	752±88.7	475±62.9** (↓34)
BWG weeks 0-13	307±53.4	321±49.4	330±47.3* (↑7)	236±41.9** (↓23)
BWG weeks 0-53	480±75.3	501±79.1	512±80.8 (↑7)	310±69.1** (↓35)
BWG weeks 0-79	514±98.5	508±105.5	567±93.3 (↑10)	286±86.3** (↓44)
BWG weeks 0-103	480±79.7	471±71.2	513±90.8 (↑7)	257±64.4** (↓46)
Week	Females			
0	181±13.6	182±14.9	179±13.6	170±14.5** (↓6)
13	312±30.3	320±34.8	310±29.1	274±25.4** (↓12)
53	440±76.3	452±86.6	430±64.1	346±56.4** (↓21)
79	495±95.8	483±81.0	483±88.1	386±60.7** (↓22)
103	488±102.1	457±98.9	483±117.7	391±52.2* (↓20)
BWG Days 0-13	131±21.5	138±24.9	131±20.2	104±15.4* (↓21)
BWG Days 0-53	260±69.8	273±78.0	253±57.3	177±47.5** (↓32)
BWG Days 0-79	315±89.6	308±75.3	307±78.1	216±53.8** (↓31)
BWG Days 0-103	309±98.2	288±96.9	305±115.3	226±48.8 (↓27)

a Data were obtained from Tables 2 and 3 on pages 7 and 8 of MRID 49779603 and Tables S35-S36 and S39-S40 on pages 255-286 and 313-336, respectively, of MRID 49779602. Percentage difference from controls is included in parentheses (calculated by reviewers).
* Significantly different ($p<0.05$) from the control group.
** Significantly different ($p<0.01$) from the control group.

Organ weights: Selected organ weights for the carcinogenicity-phase animals are presented in **Table 12.** At 0.060 mg/L, terminal body weights were decreased ($p<0.01$) by 35% in the males and ($p<0.05$) by 21% in the females. Additionally, absolute, relative (to body), and relative (to brain) lung weights were significantly increased ($p<0.01$) by 30%, 101%, and 40%, respectively, in the males, and by 23%, 53%, and 26%, respectively, in the females. Effects on lung weights in both males and females at the high concentration were associated with MITC-related microscopic findings and were, therefore, considered to be directly linked to MITC exposure.

Table 12. MITC – Sprague-Dawley [Crl:CD(SD)] Rat Study (MRID No. 49779602, 49779603) Selected mean (±SD) organ weights treated by whole-body inhalation

Dose (mg/L)	0	0.0015	0.015	0.060
ppm	0	0.5	5	20
Weight	**Males**			
Terminal body weight (g)	692±84.9	684±87.5	724±87.6	448±63.1** (↓35)
Absolute lung weight (g)	2.15±0.232	2.15±0.204	2.28±0.378	2.79±0.469** (↑30)
Relative (to body) lung weight (%)	0.314±0.0416	0.318±0.0497	0.319±0.0582	0.632±0.1260** (↑101)
Relative (to brain) lung weight (%)	94.706±8.8879	94.611±8.2469	101.537±17.2674	132.758±22.6223** (↑40)
Weight	**Females**			
Terminal body weight (g)	463±100.0	426±100.2	462±115.3	364±53.4* (↓21)
Absolute lung weight (g)	1.58±0.204	1.52±0.163	1.60±0.235	1.94±0.511** (↑23)
Relative (to body) lung weight (%)	0.351±0.0569	0.374±0.0957	0.360±0.0770	0.537±0.1379** (↑53)
Relative (to brain) lung weight (%)	76.803±9.1968	74.493±7.9201	76.667±12.4591	96.874±27.5788** (↑26)

a Data were obtained from Text Tables 11 and 12 on page 77 and Tables S59 and S60 on pages 466-475 and 476-484 of MRID 49779602. Percent difference from control were calculated by the reviewers and are included in parentheses.
* Significantly different ($p<0.05$) from the control group.
** Significantly different ($p<0.01$) from the control group.

Other evidence of toxicity: Other evidence of toxicity included bilateral keratitis in 3/58 females at 0.060 mg/L during the Week 51 examinations. This finding correlated with microscopic findings of mild acute inflammation of the corneal stroma. No treatment-related ophthalmoscopic findings were noted at lower concentrations. Decreases in percent and absolute reticulocyte counts and red blood cell (RBC) distribution width values in the high concentration males and females at Week 26. Also in the males, glucose was significantly ($p<0.01$) decreased at Weeks 26 (↓13%) and 52 (↓19%).

As earlier shown in **Tables 9** and **10**, other MITC-related findings noted in the 20 and 5 ppm males and females included olfactory epithelial degeneration and atrophy (Bowman's glands, olfactory epithelium, and olfactory nerve bundle).
CARC disagreed with the registrant regarding the adequacy of the high concentration to assess the carcinogenic potential of MITC via the inhalation route and concluded that the tumorigenic concentration was adequate and not excessively toxic for evaluating carcinogenicity, based on the following observations:

1 There was no clear evidence that the high concentration resulted in systemic toxicity or otherwise confounded observed results.
2 Although deaths were recorded in the high concentration group, there were no statistically significant differences in mortality (early or late) among the test groups throughout the study.
3 Given the high metastatic rate of the nasal tumors (and association with mortality), the CARC could not exclude the impact of tumors on body weight decreases, lung weight

increases, and clinical signs such as rales, labored respiration, and nasal discharge. Accordingly, it was concluded that tumor-associated changes in body or organ weight were not a sufficient reason to consider the high concentration to be excessively toxic.

4 The majority of the non-neoplastic findings at the high concentration were mild to moderate severity and there was no clear evidence of a distinct biological process. leading to increased mortality or other overt toxicity at the high concentration.

5 The data for the high concentration were consistent with a robust irritant but there was no overwhelming necrosis seen at the tumorigenic level (*e.g.,* generally mild to moderate necrosis was only seen in 5 male and 3 female rats at Nasal Level I).

6 Neither the study pathologist nor the peer review pathologist noted that 0.06 mg/L was an excessive concentration that may have confounded study interpretation.

The CARC, therefore, concluded that the high concentration **was not** excessive and was adequate to assess the carcinogenicity of MITC via the inhalation route of exposure.

2. Carcinogenicity study in mice

Citation: Kirkpatrick, D.T. (2015) An 18-month whole-body inhalation carcinogenicity study of methyl isothiocyanate (MITC) in mice. WIL Research, Ashland, OH. Laboratory Project ID: WIL-824014, November 13, 2015. MRID 49779601. Unpublished.

A. Experimental Design

In a carcinogenicity study (MRID 49779601), methyl isothiocyanate (MITC; 97.2-99.7% a.i.; Lot No. 56198PJV) was administered to 50 CD-1 mice/sex/concentration as a vapor by whole-body inhalation at target concentrations of 0, 1, 5, and 15 ppm (equivalent to 0.003, 0.015, or 0.045 mg/L)[4] for 6 h/day, 5 days/week for up to 78 weeks. Vapors were diluted with filtered air to achieve the target concentrations. Following 78 weeks of exposure, all surviving animals were euthanized and subjected to necropsy. All animals were observed twice daily for mortality or moribundity. Clinical observations were recorded daily and detailed physical examinations (including palpable masses) were recorded weekly. Body and food weights were recorded weekly through study week 13 and once every 2 weeks thereafter. Complete necropsies were performed on all animals and selected organs were weighed at the scheduled necropsy. Selected tissues were examined microscopically from all animals found dead or euthanized *in extremis* and from all animals in the control and high-concentration groups at the scheduled necropsy.

B. Survival

No treatment-related effect was noted on mortality, although an increasing trend ($p<0.05$) in survival was observed in males and females, with an increase ($p<0.05$) in the overall survival in the males. Survival rates at the end of the study for the 0, 0.003, 0.015, and 0.045 mg/L animals were 68%, 57%, 68%, and 88%, respectively, in males, and 74%, 83%, 81%, and 94%, respectively, in females.

4. Analytical concentrations were 0, 1.03, 5.06 and 15.11 ppm; equivalent to the above mg/L concentrations.

C. Discussion of Tumor Data

At the concentrations tested, there was no treatment-related increase in the tumor incidence compared to control. A neoplasm that consisted of a benign, exophytic[5] papilloma in nasal level I was observed in a single high-concentration female at the scheduled necropsy. The papilloma was located on the nasoturbinates in an area of MITC-related, respiratory epithelial metaplasia.

D. Adequacy of dosing

Clinical signs, body weight, ophthalmoscopic and histological findings:

At the highest concentration tested, **15 ppm,** the number of mice and occurrence of opacity of both the left and right eyes was generally increased for both sexes; body weight was significantly ($p<0.01$) decreased in males (18-21%) and females (14-16%) throughout the study **(Table 13).** Similarly, body weight gain was significantly decreased in parallel with body weight decrements with cumulative body weight gain decreases of 67-71% for males and 45-49% for females.

5 Exophytic papilloma: tumor tending to grow outward beyond the surface epithelium from which it originates.

TABLE 13. Mean (±SD) body weights and body weight gains (g) at selected intervals in mice treated with MITC by whole-body inhalation for up to 78 weeks. [a]

Weeks	Concentration (mg/L)			
	0	0.003	0.015	0.045
	ppm			
	0	1	5	15
Males				
0	30.2±1.94	30.4±2.09	30.4±1.90	30.3±1.80
13	40.3±3.01	39.8±3.52	38.2±2.51** (↓5)	33.2±2.63** (↓18)
53	44.1±3.74	43.3±3.97	41.6±2.66** (↓6)	34.9±3.25** (↓21)
77	44.0±3.94	42.6±3.46	42.4±3.22 (↓4)	34.6±3.29** (↓21)
BWG (0-13)	10.2±1.89	9.5±2.26	7.9±1.82** (↓23)	3.0±1.79** (↓71)
BWG (0-53)	14.0±2.87	13.1±3.15	11.1±2.43** (↓21)	4.6±2.20** (↓67)
BWG (0-77)	14.0±3.90	12.7±2.93	12.1±2.73* (↓14)	4.4±2.44** (↓69)
Females				
0	23.5±1.28	23.4±1.41	23.5±1.35	23.3±1.33
13	29.5±1.70	29.0±1.64	28.5±1.93** (↓3)	26.5±1.84** (↓10)
53	32.9±2.60	33.1±4.22	32.0±2.58	28.3±2.40** (↓14)
77	34.0±2.72	34.5±3.98	33.2±3.88	28.5±2.79** (↓16)
BWG (0-13)	6.0±1.47	5.6±1.25	5.1±1.59** (↓15)	3.2±1.19** (↓47)
BWG (0-53)	9.3±2.36	9.7±3.84	8.6±2.04 (↓8)	5.1±2.01** (↓45)
BWG (0-77)	10.6±2.40	11.1±3.57	9.8±3.50 (↓8)	5.4±2.18** (↓49)

a Data were obtained from Tables 7 and 8 on page 14 of MRID 49779603 and from Tables S29, S30, S31, S32, S33, and S34 on pages 182-245 of MRID 49779601. Percentage difference from controls is included in parentheses (calculated by reviewers).

* Significantly different ($p<0.05$) from the control groups

** Significantly different ($p<0.01$) from the control groups

Summarized histological findings are presented in **Table 14** (male mice) and **Table 15** (females mice). As shown, histological findings were primarily found at the high concentration (15 ppm) and to a lesser extent at the mid-concentration (5 ppm) at a lower incidence rate with minimal to mild severity grades. For example, treatment-related nasal findings at **5 ppm** included: respiratory epithelium hyperplasia (15 M; 3 F), transitional epithelial hyperplasia (15 M; 4 F), and olfactory epithelial degeneration (6F).

The above non-neoplastic findings were also noted in the **15 ppm** (**0.045 mg/L)** male and female mice with increased incidence and often increased severity. Additional findings of nasal lesions that occurred only or primarily in the 0.045 mg/L animals included: atrophy of Bowman's glands, olfactory glands, olfactory nerve bundle; degeneration of olfactory epithelium; hyperplastic changes in Bowman's glands, olfactory basal epithelium, and respiratory

epithelium; inflammation; and respiratory epithelium and squamous metaplasia. The overall incidence of the majority of these nasal lesions (across all four nasal levels) in males and/or females was 80-100%. Additional lesions that were observed in the respiratory tract of 0.045 mg/L animals included squamous metaplasia of the larynx and olfactory bulb atrophy. Inflammation of the eyes was noted in a few 0.045 mg/L animals, and was related to clinical and gross pathology findings of eye opacity.

It is of note that in a previous 13-week mouse inhalation study (MRID 48869601) with concentrations of 0, 1, 5 or 20 ppm MITC, the LOAEC for systemic effects was 5 ppm (≈ 0.015 mg/L), based on reduced body weights in male and female CD-1 mice at the LOAEC of 20 ppm (≈ 0.06 mg/L). The NOAEC for portal of entry effects was 1 ppm (≈ 0.003 mg/L), based on lesions in the nasal cavity, the larynx, and the trachea at the LOAEC of 5 ppm (≈ 0.015 mg/L). This study was cited by the registrant as providing data to establish concentrations for the mouse carcinogenicity study.

Based on the evaluation of the data, CARC concluded that the high concentration of MITC was adequate and not excessively toxic for evaluating carcinogenicity based on decreases in body weight and food consumption in both sexes and non-neoplastic, microscopic findings in the nasal levels of the respiratory tract at 0.045 mg/L.

TABLE 14. Incidence of selected non-neoplastic microscopic respiratory tract findings in male mice treated with MITC by whole-body inhalation for up to 78 weeks. [a]

Concentration (mg/L)	**0**	**0.003**	**0.015**	**0.045**
ppm	**0**	**1**	**5**	**15**
# animals	**34**	**28**	**34**	**44**
Finding	**Nasal**			
Atrophy, Bowman's glands	0	0	0	13
Atrophy, olfactory glands	0	0	0	41
Atrophy, olfactory nerve bundle	0	0	0	44
Cytomegaly/karyomegaly	0	0	1	33
Degeneration, olfactory epithelium	1	0	2	36
Exudate, inflammatory	2	1	0	29
Hyperplasia, Bowman's glands	0	0	0	44
Hyperplasia, olfactory basal epithelium	0	0	0	43
Hyperplasia, respiratory epithelium	7	8	15	32
Hyperplasia, transitional epithelium	6	3	15	43
Inflammation, chronic-active	1	1	1	34
Inflammation, submucosal glands	14	8	18	43
Metaplasia, respiratory epithelium	0	0	0	41

TABLE 14. Incidence of selected non-neoplastic microscopic respiratory tract findings in male mice treated with MITC by whole-body inhalation for up to 78 weeks. [a]

Concentration (mg/L)	**0**	**0.003**	**0.015**	**0.045**
ppm	**0**	**1**	**5**	**15**
# animals	**34**	**28**	**34**	**44**
Metaplasia, squamous	1	0	0	37
Larynx				
Hyperplasia, epithelial	0	0	0	4
Metaplasia, squamous	0	0	0	37
Exudate, luminal	1	1	3	8
Trachea				
Hyperplasia, epithelial	0	0	0	2
Olfactory bulb				
Atrophy	0	0	0	40

a Data were obtained from Table 9 on page 16 of MRID 49779603 and from Text Tables 4 and 7 on pages 64-71 and 74, respectively, of MRID 49779601.

TABLE 15. Incidence of selected non-neoplastic microscopic respiratory tract findings in female mice treated with MITC by whole-body inhalation for up to 78 weeks. [a]

Concentration	mg/L			
	0	**0.003**	**0.015**	**0.045**
	ppm			
	0	**1**	**5**	**15**
# animals	**37**	**40**	**39**	**44**
Finding	**Nasal**			
Atrophy, Bowman's glands	0	0	0	13
Atrophy, olfactory glands	0	0	0	40
Atrophy, olfactory nerve bundle	0	0	0	44
Cytomegaly/karyomegaly	0	0	1	41
Degeneration, olfactory epithelium	0	1	6	39
Exudate, inflammatory	0	1	0	25
Hyperplasia, Bowman's glands	0	0	1	44
Hyperplasia, olfactory basal epithelium	0	0	0	44
Hyperplasia, respiratory epithelium	1	4	3	25
Hyperplasia, transitional epithelium	1	5	4	41
Inflammation, chronic-active	1	0	0	33
Inflammation, submucosal glands	14	17	20	39
Metaplasia, respiratory epithelium	0	0	0	40
Metaplasia, squamous	0	0	0	35
Larynx				
Hyperplasia, epithelial	1	0	0	3
Metaplasia, squamous	0	0	0	32
Exudate, luminal	1	1	0	5
Trachea				
Hyperplasia, epithelial	1	0	0	3
Olfactory bulb				
Atrophy	0	0	0	42

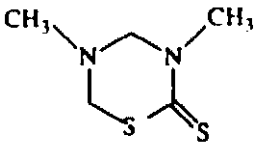
a Data were obtained from Table 9 on page 16 of MRID 49779603 and from Text Tables 4 and 7 on pages 64-71 and 74, respectively, of MRID 49779601.

IV. TOXICOLOGY

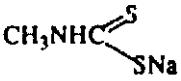
1. **Metabolism:** The only available metabolism study (MRID No. 406410-00) was in the Sprague Dawley rat via the oral route **(**See Second Report of the Cancer Assessment Review Committee, TXR No. 0055107).

2. **Mutagenicity:** There is no mutagenic concern for MITC as metam sodium via the inhalation route of exposure [See Second Report of the Cancer Assessment Review Committee (TXR No. 0055107)]. This finding is supported by the data showing that tumors occurred late in the study.**:**

3. **Structure-Activity Relationship:** Metam sodium, dazomet and MITC are related to each other in that metam sodium and dazomet are both precursors to MITC. All three metabolize to CS_2 which is a neurotoxicant but the concentration recovered only in expired air was <1%. (Cal EPA, 2003). Structures of these chemicals are as follows:



Dazomet



Metam Sodium

Methyl isothiocyanate (MITC)

For details **(**See Second Report of the Cancer Assessment Review Committee (TXR No. 0055107).

4. **Subchronic and Chronic Toxicity:**

Subchronic Inhalation Toxicity in Rats

In a 28-day inhalation toxicity study (MRID 45314802), Methyl Isothiocyanate [96.9 % a.i.] was administered to 5/sex/dose of SPF Wistar/Chubb:THOM rats by whole body exposure at analytical concentrations of 0, 5.0, 20, or 100 mg/m^3 equivalent to 0, 5.0, 20, or 100 µg/L (measured concentrations 0, 5.1, 19.9 or 100 µg/L) for 6 hours per day, 5 days/week for a total of 28 days.

All animals survived to study termination. Mid and high dose rats demonstrated clinical signs during exposure from the third exposure period onward. In the high dose rats, the signs persisted during the non-exposure periods. Body weight and body weight gain were significantly decreased ($p < 0.05$) at the high dose. Food consumption and feed efficiency were not measured. There was an increase in serum bilirubin that was statistically significant ($p < 0.01$) in the high dose males. The biological significance of the increase is unknown. There was increased lung weight, accompanied by bronchopneumonia, as well as other gross and microscopic changes in the respiratory tract of high dose male and female rats including, but not limited to, atrophy of the olfactory epithelium; tracheal cell necrosis, and focal squamous cell metaplasia in the respiratory epithelium.

In a 90-day inhalation (MRID 00162041), 18 Sprague-Dawley rats/sex/dose group were exposed to aerosolized metam sodium (37% a.i.) in whole-body chambers for 6 hr/day, 5 days/week. The

cumulative mean chamber metam sodium concentrations were 0, 6.5, 45 and 160 mg/m^3 (measured values based on the sodium ion level corrected for sodium ion levels measured from the control). Reviewers at the California Department of Pesticide Regulation calculated the doses to be 0, 1.11, 7.71, and 27.43 mg/kg/day. Mean MITC measured concentrations were 0, 0.78, 2.2, and 5.7 mg/ m^3 (0, 0.12, 0.38, 0.98 mg/kg/day) (measured by infrared adsorption).

Clinical signs of salivation, dullness, chromodacryorrhea, dehydration, rough coat, and wet coat were noted in males and females of the highest concentration level. There was no treatment related mortalities.

Body weight gain was reduced at the highest concentration level compared to control (- 6% and - 8% for males and females, respectively). Food consumption was decreased compared to control in the mid and highest levels (-8% and - 10%).

At the interim measurement, plasma lactate dehydrogenase levels were statistically reduced by 50% and 62% ($p< 0.05$) in females in the mid (7.71 mg/kg/day, metam sodium) and high (27.43 mg/kg/day, metam sodium) doses, respectively, but only at the highest dose in males (-18%). At termination, albumin was decreased compared to control (-13% and -22%; $p<0.05$) and alkaline phosphatase increased (+2- fold; $p<0.05$) at the mid and high doses, respectively, in females only.

Although the absolute weights were not affected, significant increases in relative lung (+13% males, +21% females) and kidney (+7% males, +14% females) weights were noted in the highest dose group.

Histopathology indicative of irritation was noted in the nasal passages, lung, and stomach. A dose-dependent increase in the incidence of mucigenic hyperplasia of the nasal passage was noted in all treatment groups for females but only reached statistical significance in the mid and high dose group. This finding (*i.e.,* incidence of mucigenic hyperplasia) was increased ($p<0.05$) only in the male high dose group. Mucigenic cysts were noted in 2 females of the highest dose group. A dose-dependent increase in lymphocytic rhinitis was noted in all treatment groups although statistical significance was noted only at the mid and high dose males. In the lungs, histiocytosis was noted in 3/27 high dose males and 2/18 high dose females. In the stomach, erosive gastritis was statistically increased in the high dose males and females (9/17 males, 13/18 females). Ulcerative gastritis was noted in 2/18 high dose females. Gross pathological changes in stomach were also noted at the high dose in males and females by in an increased incidence in red/black foci or streaks.

In a second 13-week whole-body inhalation toxicity study (MRID 48869801) methyl isothiocyanate (MITC; Lot No. 56198PJV; 99.7% purity) vapor was administered to Sprague-Dawley rats (10/sex/concentration) for 6 hr/day, 5 days/wk at exposure concentrations of 0, 1, 5, or 15 ppm (≈ 0, 0.003, 0.015, or 0.05 mg/L, respectively). Test material concentrations were prepared by varying the vapor flow rates through the transvector jets of the exposure chamber and mixing with an appropriate volume of filtered air. Clinical signs were observed daily; body weight and food consumption were recorded weekly, and blood samples were collected at the scheduled sacrifice for hematology and clinical chemistry. Selected organs were weighed on all animals and designated tissues were examined microscopically.

All animals survived until scheduled sacrifice. No treatment-related clinical findings were observed. There was no statistically significant effect on body weight or body weight gain of male or female rats at any MITC exposure concentration. Higher mean absolute neutrophil count (+57%; $p<0.01$) and a higher thromboplastin time (+18%; $p<0.05$) were observed in the high dose females, but no microscopic correlates were found and these observations were not seen in males. There were no test substance-related macroscopic changes observed that were associated with MITC exposure. There were no statistically significant or biologically significant organ weight changes observed that were associated with MITC exposure. Microscopic alterations were, however, noted in the nasal cavity in the 1 ppm group males and in both males and females at 5 and 15 ppm. Lesions were also noted in the larynx in males of the 5-ppm group and in both males and females at 5 and 15 ppm. Nasal epithelial findings (in respiratory, transitional, and/or olfactory epithelium) were noted in all 15-ppm group males and females. Epithelial findings were rare in the 1 and 5 ppm groups. Acute inflammation in nasal Level IV in animals exposed to 15 ppm was also noted. Minimal acute inflammation was observed in nasal Level II in females in the 5-ppm group. In the larynx, epithelial findings were observed in the majority of males and females in the 15-ppm group, but in only 2 males in the 5-ppm group. Test substance-related changes in the larynx were limited to minimal to mild single cell necrosis and epithelial regeneration, which were characterized as scattered individual necrotic cells with pyknotic nuclei and hyper-eosinophilic cytoplasm.

Subchronic Inhalation Toxicity in Mice

In a 13-week whole-body inhalation toxicity study (MRID 48869601) methyl isothiocyanate (MITC; Lot No. 56198PJV; 99.7% purity) vapor was administered to CD-1 mice (10/sex/concentration) for 6 hr/day, 5 days/week at exposure concentrations of 0, 1, 5, or 20 ppm (equivalent to ≈0, 0.003, 0.015, or 0.06 mg/L, respectively). Test material concentrations were prepared by varying the vapor flow rates through the transvector jets of the exposure chamber and mixing with an appropriate volume of filtered air. Clinical observations, body weight, and food consumption were made daily and/or weekly throughout the study. Blood samples for hematology and clinical chemistry, as well as selected organs that were weighed, were collected from all animals. Designated tissues were examined microscopically.

Two animals died at the 1 ppm exposure prior to scheduled necropsy (deaths were unrelated to MITC exposure). MITC-related clinical observations seen at the midpoint of exposure at 5 and 20 ppm included hypoactivity, hyperactivity, complete and/or partial closure of the eyes, and standing posture during exposure, and labored respiration. These findings were transient in nature and did not persist following exposures. Test substance-related lower body weight was noted in the 20-ppm group males and females throughout the study (11% lower in males, 13% lower in females). MITC-related food consumption was consistently lower by about one-third in the 20-ppm group males and females throughout the study ($p<0.01$). Lower mean liver and spleen weights ($p<0.05$ and $p<0.01$, respectively), associated with MITC exposure, were observed in the 20-ppm group males and females. The changes in spleen weight were not considered adverse because there were no corresponding microscopic findings. Minimal to mild decreased glycogen was noted in the livers of the 20-ppm group males and females. Nasal lesions were observed in all nasal sections of the 20-ppm group males and females; the most

prominent findings in all nasal levels (Levels I-VI) included: squamous epithelial hyperplasia, hyperkeratosis, metaplasia, cytomegaly / karyomegaly, erosion, inflammation, and exudates. Mice in the 5-ppm group had a subset of the lesions with a much lower incidence and severity. MITC-related microscopic findings of the larynx included minimal single cell necrosis of widely scattered epithelial cells accompanied by minimal hyperplasia in the 5-ppm group males and 20-ppm group males and females. The most prominent findings at all nasal levels (I - VI) were hyperplasia, hyper-keratosis, metaplasia, cytomegaly/karyomegaly, erosion, inflammation, and exudates. In the trachea, there was single cell necrosis in widely scattered epithelial cells in low numbers of the 5-ppm group males and 20-ppm group males and females. Two female mice from the 20-ppm group had single cell necrosis in the bronchi of the lungs.

Chronic Inhalation Toxicity:

Chronic Toxicity/Carcinogenicity Inhalation Study in Rats

In a combined chronic toxicity/carcinogenicity study (MRID 49779602), methyl isothiocyanate (MITC; Lot # 56198PJV; 97.2-99.7% a.i.) was administered to 60 Sprague Dawley [Crl:CD(SD)] rats/sex/dose by whole-body inhalation at nominal concentrations of 0, 0.5, 5 and 20 ppm (equivalent to 0, 0.001, 0.015, or 0.060 mg/L)[6] diluted with filtered air for 6 h/day, 5 days/week for 104 weeks.

All animals were observed twice daily for mortality or moribundity. Clinical observations were recorded daily and detailed physical examinations (including palpable masses) were recorded weekly. Body weights were recorded weekly through study week 13 and once every 2 weeks, thereafter. Food consumption was recorded weekly through study week 13 and at least 1 week per month, thereafter. Ophthalmic examinations were performed during study weeks -1 and 51. Clinical pathology parameters (hematology, coagulation, serum chemistry, and urinalysis) were collected from all animals assigned to the Chronic Phase of the study at approximately 6 months (excluding coagulation parameters; study week 26) and on the day of their scheduled interim necropsy (study week 52). Blood smears were prepared from all Carcinogenicity Phase animals euthanized *in extremis* (if possible) and from all surviving animals at the scheduled necropsy (study week 104). Complete necropsies were performed on all animals and selected organs were weighed at the scheduled necropsies. Selected tissues were examined microscopically from all animals found dead or euthanized *in extremis* and from all animals in the control and high-concentration groups at the scheduled necropsies.

Chronic phase

There were no adverse, treatment-related effects observed on urinalysis or gross pathology at any concentration. Although there were no significant differences in survival during the chronic or the carcinogenicity phases of testing, deaths in the high concentration group were associated with neoplastic lesions of the nose and associated tissues. Findings for other parameters at different concentrations were as follows:

At **0.001mg/L**, there were no treatment-related effects on clinical signs, body weight, food consumption or food efficiency, ophthalmoscopic examinations, hematology or clinical chemistry, organ weights or gross pathology. In the combined nasal regions of the males, microscopic findings included: minimal to mild metaplasia squamous (20 vs. 8 in control) in the

[6]Analytical concentrations were 0, 0.5, 4.83 and 19.87 ppm; equivalent to the above mg/L concentrations.

males and degeneration of the olfactory epithelium (5 vs. 1 in control) and minimal to mild, with one severe transitional epithelial hyperplasia (40 vs. 23 in control) in the females. In the larynx of the males: minimal to mild epithelial hyperplasia (17 vs. 3 in control) and minimal to mild squamous hyperplasia (6 vs. 0 in control) was observed. In the females, minimal to mild, with one moderate epithelial hyperplasia (28 vs. 8 in control) was seen. It is of note that all of these findings occurred in a dose-related manner for all concentrations and the degree of severity generally followed a pattern that was similar to the mid-concentration group.

At **0.015 mg/L**, rales and labored respiration were noted during clinical observations. Body weight, food consumption organ weight or gross pathology were unaffected by treatment. Microscopic findings were: olfactory epithelia degeneration (47/60M; 43/60F), atrophy [Bowman's glands (28/60M; 23/60F)], olfactory nerve bundles (32/60M; 34/60F)], hyperplasia [transitional epithelial (59/60M; 52/60F), respiratory epithelial/mucous cell (50/60M; 44/60F)], metaplasia [respiratory epithelial (3/60M; 4/60F); squamous (38/60M; 19/60F)] and chronic-active inflammation (53/60M; 51/60F) were recorded in the nasal region. In addition, squamous epithelial hyperplasia (32/60M; 37/60F) in the larynx. The above findings were considered adverse.

At **0.060 mg/L**, the following findings were noted during clinical observations: increased incidence of rales, labored respiration, clear and red nasal discharge, red material around the nose (males only), and left and right eye opacities. Treatment-related palpable masses on or near the nose were found and were associated with neoplastic findings observed in this area.

Body weights: Body weights were decreased consistently throughout treatment by 8-35% in the males, and by 6-26% in the females at 0.060 mg/L. Similarly, cumulative body weight gains were generally decreased throughout the study, with overall (Weeks 0-103) body weight gains decreased by 46% and 27% in the males and females, respectively. Decreased food consumption values were generally observed throughout treatment that correlated with the decreased body weights. Slightly decreased food efficiency was also generally noted in this group.

Ophthalmoscopic findings: Bilateral keratitis was observed in 3/58 females at 0.060 mg/L during the Week 51 examinations. This finding correlated with microscopic findings of mild acute inflammation of the corneal stroma.

Hematological and clinical chemistry findings: Hematological and clinical chemistry findings were confined to the 0.060 mg/L group and included: significant ($p<0.01$) decreases in percent and absolute reticulocyte counts and red blood cell (RBC) distribution width values in the males and females at Week 26. At Week 52, these parameters were no longer significant but had increased in magnitude. In the females at Week 26, increases were noted in white blood cells (WBC), neutrophils, lymphocytes, monocytes, and basophils consistent with an inflammatory response in the nasal tissues. Also in the males, glucose was significantly ($p<0.01$) decreased at Weeks 26 (↓13%) and 52 (↓19%).

Organ weights: In the chronic-toxicity phase animals, terminal body weights of the high concentration groups were decreased by 23% in the males and by 19% in the females. Additionally, absolute, relative (to body), and relative (to brain) lung weights were increased by 8%, 41%, and 14%, respectively, in the males, and by 11%, 37%, and 15%, respectively, in the females. In the carcinogenicity-phase animals, terminal body weights were decreased by 35% in the males and by 21% in the females. Additionally, absolute, relative (to body), and relative (to

brain) lung weights were increased by 40%, 101%, and 40%, respectively, in the males, and by 21%, 53%, and 26%, respectively, in the females.

Non-and pre-neoplastic findings: Extensive, microscopic, non-and pre-neoplastic findings were observed in the nasal cavity (levels I through VI), larynx, trachea, lungs, olfactory bulbs, and eyes. These findings, which were generally mild to moderate, increased in incidence and severity with increasing concentrations, are listed below for the high concentration group:

Nasal cavity: atrophy of Bowman's glands (57M/56F); olfactory epithelia degeneration (33/60M; 46/60F); atrophy of olfactory nerve bundle (58M/56F); atrophy of the olfactory epithelia (58/60M; 56/60F); inflammatory exudate (60M/60F); hyperplasia of the respiratory epithelial/mucous cell (60/60M; 59/60F); hyperplasia of the squamous epithelia (54/60 M; 55/60 F); transitional epithelial hyperplasia (44/60 M; 47/60 F); chronic-active inflammation (59/60 M; 59/60 F); metaplasia of the respiratory epithelia (60/60 M; 58/60 F); squamous metaplasia (60/60 M; 58/60 F); and mineralization (9/60 M; 10/60 F).

Level I: hyperplasia of the squamous epithelia (54/60 M; 55/60 F).

Level II: hyperplasia of the respiratory epithelia/mucous cell (51/60 M; 44/60 F); transitional epithelial hyperplasia (44/60 M; 47/60 F); squamous metaplasia (59/60 M; 60/60 F).

Level III: degeneration of the olfactory epithelium (3/60 M; 2/60 F); hyperplasia of the respiratory epithelia/mucous cell (55/60 M; 55/60 F); metaplasia of the respiratory epithelium (55/60 M; 41/60 F); squamous metaplasia (59/60 M; 54/60 F).

Level IV: degeneration of the olfactory epithelium (9/60 M; 20/60 F); hyperplasia of the respiratory epithelia/mucous cell (55/60 M; 55/60 F); metaplasia of the respiratory epithelium (55/60 M; 52/60 F); squamous metaplasia (50/60 M; 49/60 F).

Level V: degeneration of the olfactory epithelium (27/60 M; 41/60 F); hyperplasia of the respiratory epithelia/mucous cell (57/60 M; 54/60 F); metaplasia of the respiratory epithelium (54/60 M; 55/60 F); squamous metaplasia (40/60 M; 30/60 F).

Level VI: degeneration of the olfactory epithelium (21/60 M; 26/60 F); hyperplasia of the respiratory epithelia/mucous cell (55/60 M; 56/60 F); metaplasia of the respiratory epithelium (48/60 M; 32/60 F); squamous metaplasia (50/60 M; 49/60 F); squamous metaplasia (13/60 M; 5/60 F); degeneration of the olfactory epithelium (18/60 M; 9/60 F).

Larynx: epithelial hyperplasia (29/60 M; 32/60 F); squamous metaplasia (60/60 M; 60/60 F); inflammatory exudate ((3/60 M; 2/60 F); and regeneration of the respiratory epithelia (10/60 M; 10/60 F).

Trachea: hyperplasia of the respiratory epithelia (41/60 M; 32/60 F); mixed cell inflammation (10/60 M; 3/60 F); squamous metaplasia (3/60 M; 4/60 F); and regeneration of the respiratory epithelia (23/60 M; 34/60 F).

Lungs: luminal exudate (29/60 M; 20/60 F); fibrosis (39/60 M; 47/60 F); pleural fibrosis (6/60 M; 1/60 F); mucous cell hyperplasia (10/60 M; 3/60 F); hyperplasia of the respiratory epithelia (45/60 M; 44/60 F); chronic inflammation (18/60 M; 14/60 F); alveolar macrophages (44/60 M; 41/60 F); squamous metaplasia (12/60 M; 4/60 F); regeneration of the respiratory epithelia (41/60 M; 46/60 F); and ulceration (29/60 M; 32/60 F).

Olfactory bulbs: atrophy of the olfactory bulbs (41/60 M; 49/60 F), which was stated to be secondary to atrophy of the olfactory epithelia and nerve bundles in nasal level VI, was characterized by loss of the olfactory nerve layer and occasional thinning of the glomerular layer.

In the eyes, epithelial hyperplasia of the corneal epithelia (1/35 F vs. 0/34 in control) and lens degeneration (1/37 M vs. 0/39 in control) were observed at 0.015 mg/L. At 0.06 mg/L, acute inflammation of the corneal stroma (4/35 F vs. 0/34); subacute inflammation of the corneal stroma (13/37 M vs. 0/39 in control; 9/35 F vs. 0/34 in control); hyperplasia of the corneal epithelia (2/35 F vs. 0/34 in control); and lens deterioration (2/37 M vs. 0/39 in control) was reported.

Neoplastic findings: Neoplastic findings were observed in the nasal cavity, with extensions of these tumors into various nasal levels, the adjacent bone, brain, gingiva, hard palate, and skin or metastases to the lungs or lymph nodes at the highest concentration only.

Carcinogenicity Inhalation Study in Mice

In a carcinogenicity study (MRID 49779601), methyl isothiocyanate (MITC; 97.2-99.7% a.i.; Lot No. 56198PJV) was administered to 50 CD-1 mice/sex/concentration as a vapor by whole-body inhalation at target concentrations of 0, 1, 5 and 15 ppm (equivalent to 0.003, 0.015, or 0.045 mg/L)[7] for 6 h/day, 5 days/week for up to 78 weeks. Vapors were diluted with filtered air to achieve the target concentrations.

There were no effects of treatment on mortality/survival (an increasing trend ($p<0.05$) in survival was observed in both sexes, with an increase ($p<0.05$) in overall survival in males), organ weights, or neoplastic findings

Clinical findings were confined to ocular effects, including opacity, which was correlated with macroscopic findings of eye opacity in the high concentration males and females. Significant, ≥10% decreased in body weight were also noted in both sexes of the 0.045 mg/L group, commencing at week 2 and throughout the study for the males and starting in week 10 and throughout the remainder of the study in females. Body weight gain was also significantly decreased by ≥66% (M) and ≥41% (F) at this level. Significant but <10 % decreases were also see at 0.015 mg/L in both sexes accompanied by ≈ 20% ↓ in male body weight gain and ≈ 10% ↓ in female body weight gain. However, body weights at the mid-concentration never achieved ≥10% decrement, therefore, in keeping with current HED practices, the weight reductions at 0.015 mg/L were not considered adequate evidence of an adverse effect. Significant ($p<0.01$) reductions in food consumption were generally observed for all intervals of the high concentration males and females and ranged from 11 to 21% decreases (males) and 14 to 24% decreases (females).

Organ Weights: Significantly ($p<0.01$) decreased liver (absolute, relative to body, and relative to brain weights) were noted in the 0.045 mg/L males and females. Absolute, relative to body, and relative to brain weights were decreased by 34%, 16% and 30% in the males, respectively and by 25%, 11% and 20% in the females, respectively. Significant and/or marked decreases in absolute, relative to body, and relative to brain weights were also noted for the spleen and

7 Analytical concentrations were 0, 1.03, 5.06 and 15.11 ppm; equivalent to the above mg/L concentrations.

thymus of the high concentration males and females. However, none of the organ weight decrements were supported by microscopic findings; therefore, these findings are not considered adequate evidence of an adverse effect.

Non-neoplastic findings: Treatment-related, non-neoplastic microscopic findings were limited to the respiratory tract (nasal levels, larynx, trachea, and olfactory bulb) and the eyes. At **0.015 mg/L**, MITC-related nasal findings occurred with low incidence and severity (minimal to mild) in a few regions. Treatment-related nasal findings included transitional epithelial hyperplasia (males only), olfactory epithelial degeneration (females > males), and olfactory nerve bundle atrophy and Bowman's gland hyperplasia (females only). Transitional epithelial hyperplasia occurred in 0.015 mg/L males with increased incidence (24/50 vs. 7/50 control), and lesions were located on the naso- and maxillary turbinates, as well as the lateral and middle meatuses. Olfactory epithelial degeneration was noted with a greater incidence in females (9/50 females vs. 3/50 males). Although the incidence was low, the degeneration was considered to be treatment-related at this concentration due to the distribution of the lesion in the dorsal meatus, septum, and ethmoid turbinates). The incidence of olfactory nerve bundle atrophy and Bowman's gland hyperplasia in females was minimal (n = 2/50 and 1/50, respectively), but was considered to be related to treatment because the incidence of both findings at the next highest concentration (0.045 mg/L) was 100% (50/50) for both sexes.

The above non-neoplastic findings were also noted in the **0.045 mg/L** animals with increased incidence and often increased severity. Additional findings of nasal lesions that occurred only or primarily in the 0.045 mg/L animals included: atrophy of Bowman's glands and olfactory epithelium; dilatation; hyperplastic changes in Bowman's glands, olfactory basal epithelium, and respiratory epithelium; inflammation/exudation; respiratory epithelial and squamous metaplasia; necrosis; and ulceration. The overall incidence of the majority of these nasal lesions (across all four nasal levels) in males and/or females was 80-100%. Additional lesions that were observed in the respiratory tract of 0.045 mg/L animals included epithelial hyperplasia of the larynx and trachea, squamous metaplasia of the larynx, and atrophy of the olfactory bulb. Squamous metaplasia of the larynx and olfactory bulb atrophy were the primary effects with the incidence rate ranging from 66-91%. Inflammation of the eyes was noted in a few 0.045 mg/L animals, and was related to clinical and gross pathology findings of eye opacity.

VI. MODE of ACTION STUDIES

No mode of action studies for tumor formation have ever been submitted to HED.

VII. COMMITTEE'S ASSESSMENT OF THE WEIGHT-OF-THE-EVIDENCE

1. Carcinogenicity

Rat

Male and female rats had statistically significant trends and showed significant pair-wise comparisons for nasal carcinomas at the high dose (20 ppm; 0.06 mg/L) compared to the controls.

The CARC determined that the high test concentration was adequate and not excessively toxic for evaluating carcinogenicity, based on the following observations:

1. There was no clear evidence that the high concentration resulted in systemic toxicity or otherwise confounded observed results.
2. There were no statistically significant differences in early mortality among the treatment groups throughout the study.
3. Given the high metastatic rate of the nasal tumors (and association with mortality), the CARC could not exclude the impact of tumors on body weight decreases and lung weight increases and clinical signs such as rales, labored respiration, and nasal discharge. Accordingly, it was concluded that tumor-associated changes in body or organ weight were not a sufficient reason to consider the high concentration to be excessively toxic.
4. The majority of the non-neoplastic findings at the high concentration were mild to moderate severity, and there was no clear evidence of a distinct biological process leading to increased mortality or other overt toxicity at the high concentration.
5. The data for the high concentration were consistent with a robust irritant effect, but there was no overwhelming necrosis seen at the tumorigenic level (*e.g.,* generally mild to moderate necrosis was only seen in 5 male and 3 female rats at Nasal Level I).
6. Neither the study pathologist nor the peer review pathologist noted that 0.06 mg/L was an excessive concentration that may have confounded study interpretation.

The CARC concluded that the nasal tumors in male and female rats are treatment-related.

Mouse

There were no treatment-related tumors in male or female mice.

2. Mutagenicity
In agreement with the earlier findings, mutagenicity is not a concern for MITC.

3. Structure Activity Relationship
Metam sodium and dazomet are both precursors to MITC. For details **(**See Second Report of the Cancer Assessment Review Committee (TXR No. 0055107).

VIII. CLASSIFICATION OF CARCINOGENIC POTENTIAL

In accordance with the EPA's Final Guidelines for Carcinogen Risk Assessment (March 2005), the CARC classified MITC via the inhalation route as "Likely to be Carcinogenic to Humans" based on treatment-related nasal tumors in both sexes in the rat.

IX. QUANTIFICATION OF CARCINOGENIC POTENTIAL

The CARC recommended a linear low-dose extrapolation model (Q1*) for human cancer risk assessment.

X. BIBLIOGRAPHY

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